

To contribute to the establishment of a generalized spectral approach for vegetation stress detection, this study compares the ability of high-spectral-resolution reflectance (R) and fluorescence (F) foliar measurements to detect vegetation changes associated with common environmental factors affecting plant growth and productivity. To obtain a spectral dataset from a broad range of species and stress conditions, plant material from three experiments was examined, including (i) corn, nitrogen (N) deficiency/excess; (ii) soybean, elevated carbon dioxide, and ozone levels; and (iii) red maple, augmented ultraviolet irradiation; measuring Fluorescence and R spectra (400–800 nm) on the same foliar samples in conjunction with photosynthetic pigments, carbon, and N content. For separation of a wide range of treatment levels, hyperspectral (5–10 nm) R indices were superior compared with F or broadband R indices, with the derivative parameters providing optimal results. For the detection of changes in vegetation physiology, hyperspectral indices can provide a significant improvement over broadband indices. The relationship of treatment levels to R was linear, whereas that to F was curvilinear, therefore using reflectance measurements, it was not possible to identify the unstressed vegetation condition, which was accomplished in all three experiments using F indices. Large-scale monitoring of vegetation condition and the detection of vegetation stress could be improved by using hyperspectral R and F information, a possible strategy for future remote sensing missions.

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## 2) Title: Contribution of Chlorophyll Fluorescence to the Apparent Reflectance of Vegetation

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### Abstract

Current strategies for monitoring the physiologic status of terrestrial vegetation rely on remote sensing reflectance (R) measurements, which provide estimates of relative vegetation vigor based primarily on chlorophyll content. Vegetation chlorophyll fluorescence (CF) offers a non-destructive alternative and a more direct approach for diagnosis of vegetation stress before a significant reduction in chlorophyll content has occurred. Thus, monitoring of vegetation vigor based on CF may allow earlier stress detection and more accurate carbon sequestration estimates, than is possible using R data alone. However, the observed apparent vegetation reflectance (Ra) in reality includes contributions from both the reflected and fluoresced radiation. The aim of this study is to determine the relative R and CF fractions contributing to Ra from the vegetation in the red to near-infrared region of the spectrum. The practical objectives of the study are to: 1) evaluate the relationship between CF and R at the foliar level for corn, soybean, maple,; and 2) for corn, determine if the relationship established for healthy (optimal N) vegetation changes under N deficiency. To obtain generally applicable results, experimental measurements were conducted on unrelated crop and tree species (maple, soybean and corn), under controlled conditions and a gradient of inorganic N fertilization levels. Optical R spectra and actively induced CF emissions were obtained on the same foliar samples, in conjunction with measurements of photosynthetic function, pigment levels, and C and N content. The common spectral trends or similarities were examined. On average, 10-20% of apparent R at 685 nm was actually due to CF. The spectral trends in steady and maximum F varied significantly, with Fs (especially red) showing higher ability for species and treatment separation. The relative contribution of ChlF to R varied significantly among species, with maple emitting much higher F amounts, as compared to corn and soybean. Fs individual red and far-red bands and their ratio exhibited consistent species separations. For

corn, the relative CF fraction increased in concert with the nutrient stress levels from <2% for non-stressed foliage to >7% for severely nutrient deficient plants. F685s provided optimal treatment separation. This study confirms the trends in F685s/F740s associated with N deficiency and vegetation stress, established using single narrow band excitation.

Popular summary

Vegetation chlorophyll fluorescence (CF) offers a non-destructive alternative and a more direct approach for diagnosis of vegetation stress before a significant reduction in chlorophyll content has occurred. Monitoring of vegetation vigor based on CF may allow earlier stress detection and more accurate carbon sequestration estimates, than is possible using R data alone. However, the observed apparent vegetation reflectance (Ra) in reality includes contributions from both the reflected and fluoresced radiation. The aim of this study is to determine the relative R and CF fractions contributing to Ra from the vegetation in the red to near-infrared region of the spectrum. To obtain generally applicable results, experimental measurements were conducted on crop and tree species (maple, soybean and corn), under controlled conditions and a gradient of inorganic N fertilization levels. Optical R spectra and actively induced CF emissions were obtained on the same foliar samples, in conjunction with measurements of photosynthetic function, pigment levels, and C and N content. On average, 10-20% of apparent R at 685 nm was due to CF. The relative contribution of ChlF to R varied significantly among species, with maple emitting much higher F amounts, as compared to corn and soybean. F's individual red and far-red bands and their ratio allowed consistently species separation. For corn, the relative CF fraction increased in concert with the nutrient stress levels from <2% for non-stressed foliage to >7% for severely nutrient deficient plants. F685s provided optimal treatment separation. This study confirms the trends in F685s/F740s associated with N deficiency and vegetation stress, established using single narrow band excitation.

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Subject: Publications  
Cc: Sandi\_Bussard, Betsy Middleton <Elizabeth.M.Middleton@nasa.gov>  
Bcc:  
Attached:

Dear Mr. Cole,

Please find attached the abstracts and other pertaining information of two of my publications.  
Thank you for your consideration.

Regards,

Petya Campbell

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1) **Title:** Assessment of Vegetation Stress Using Reflectance or Fluorescence Measurements

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**Abstract**

Current methods for large-scale vegetation monitoring rely on multispectral remote sensing, which has serious limitation for the detection of vegetation stress. To contribute to the establishment of a generalized spectral approach for vegetation stress detection, this study compares the ability of high-spectral-resolution reflectance (R) and fluorescence (F) foliar measurements to detect vegetation changes associated with common environmental factors affecting plant growth and productivity. To obtain a spectral dataset from a broad range of species and stress conditions, plant material from three experiments was examined, including (i) corn, nitrogen (N) deficiency/excess; (ii) soybean, elevated carbon dioxide, and ozone levels; and (iii) red maple, augmented ultraviolet irradiation. Fluorescence and R spectra (400–800 nm) were measured on the same foliar samples in conjunction with photosynthetic pigments, carbon, and N content. For separation of a wide range of treatment levels, hyperspectral (5–10 nm) R indices were superior compared with F or broadband R indices, with the derivative parameters providing optimal results. For the detection of changes in vegetation physiology, hyperspectral indices can provide a significant improvement over broadband indices. The relationship of treatment levels to R was linear, whereas that to F was curvilinear. Using reflectance measurements, it was not possible to identify the unstressed vegetation condition, which was accomplished in all three experiments using F indices. Large-scale monitoring of vegetation condition and the detection of vegetation stress could be improved by using hyperspectral R and F information, a possible strategy for future remote sensing missions.

**Popular summary**

# Contribution of Chlorophyll Fluorescence to the Apparent Reflectance of Vegetation

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## Abstract

Current strategies for monitoring the physiologic status of terrestrial vegetation rely on remote sensing reflectance (R) measurements, which provide estimates of relative vegetation vigor based primarily on chlorophyll content. Vegetation chlorophyll fluorescence (CF) offers a non-destructive alternative and a more direct approach for diagnosis of vegetation stress before a significant reduction in chlorophyll content has occurred. Thus, monitoring of vegetation vigor based on CF may allow earlier stress detection and more accurate carbon sequestration estimates, than is possible using R data alone. However, the observed apparent vegetation reflectance (Ra) in reality includes contributions from both the reflected and fluoresced radiation. The aim of this study is to determine the relative R and CF fractions contributing to Ra from the vegetation in the red to near-infrared region of the spectrum. The practical objectives of the study are to:

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1) evaluate the relationship between CF and R at the foliar level for corn, soybean, maple,; and 2) for corn, determine if the relationship established for healthy (optimal N) vegetation changes under N deficiency.

To obtain generally applicable results, experimental measurements were conducted on unrelated crop and tree species (maple, soybean and corn), under controlled conditions and a gradient of inorganic N fertilization levels. Optical R spectra and actively induced CF emissions were obtained on the same foliar samples, in conjunction with measurements of photosynthetic function, pigment levels, and C and N content. The common spectral trends or similarities were examined. On average, 10-20% of apparent R at 685 nm was actually due to CF. The spectral trends in steady and maximum F varied significantly, with Fs (especially red) showing higher ability for species and treatment separation. The relative contribution of ChlF to R varied significantly among species, with maple emitting much higher F amounts, as compared to corn and soybean. Fs individual red and far-red bands and their ratio exhibited consistent species separations. For corn, the relative CF fraction increased in concert with the nutrient stress levels from <2% for non-stressed foliage to >7% for severely nutrient deficient plants. F685s provided optimal treatment separation. This study confirms the trends in F685s/F740s associated with N deficiency and vegetation stress, established using single narrow band excitation.

Key words: Vegetation function/stress, Remote sensing, Apparent vegetation reflectance, Chlorophyll fluorescence, High resolution spectra

## 1. INTRODUCTION

Current remote sensing techniques for monitoring the status of terrestrial vegetation rely on reflectance (R) data to estimate chlorophyll content and stand structure in order to infer vegetation vigor. However, R observations are not able to directly assess vegetation photosynthetic function and physiological status. Improvements in satellite-based assessments of the terrestrial vegetation's carbon budget require additional information on vegetation physiological condition, which can be obtained *directly* by fluorescence (F) emissions. Considering the rapid developments in the technology: 1. signal amplification and sensor miniaturization, 2. laser induced fluorescence technologies and 3. passive chlorophyll F sensing technologies, utilizing the Fraunhofer line depth approach, F has strong practical potential for monitoring vegetation status.

The red/far-red region the apparent vegetative reflectance (Ra) typically includes the contribution of both the R and F, emitted from the foliage surface. A portion of the absorbed solar energy is utilized in the process of photosynthesis via biochemical reactions, while the absorbed energy not utilized in the photosynthesis is either emitted as F at longer wavelengths or dissipated as heat. Vegetation F is emitted from the foliage throughout the ultraviolet to visible regions of the spectrum, with peaks occurring at 320, 445, 530, 685, and 740 nm. While it has been assumed that F is a very small portion of Ra, further research is needed to quantify the contribution of F to Ra, especially in the red (685nm) and far-red (740nm) where the ChF emissions are maximal.

F spectral indices, derived using active ChlF in the red and far-red, have successfully detected various types of vegetation stress, and quantified the amount of crop residue covering agricultural soil surfaces. Active laser induced F technologies

apply a strong narrow pulsed excitation beam of illumination and differentiate F emission from R through fast gated detector technologies. The amount of the emitted F strongly depends on the wavelength and amount of the excitation energy. The photosynthetic pigments absorb radiation primarily in the 350-700 nm region, while their emittance occurs in broad peaks in the red (F685) and far-red (F740) portions of the spectrum. While active F techniques have established ChlF as a reliable approach for diagnosis of vegetation physiological condition, very few studies validate the potential of solar induced ChlF.

The goals of this investigation are, using as excitation the full solar spectrum to: 1) determine the relative R and ChlF fractions contributing to the cumulative vegetation irradiance at 685 nm, 740 nm and 760 nm (contribution of ChlF to Ra) at the foliar level, and 2) examine the variation in ChlF associated with species differences or in corn, nitrogen (N) deficiency.

## 2. METHODS

### 2.1 Vegetation material and treatments

Experimental measurements were acquired in 2001 and 2004 at the middle of the growing season at the several field sites at the USDA Agricultural Research Center, Beltsville, MD. To simulate the varying rates of total seasonal atmospheric nitrogen (N) deposition and provide a range in plant growth conditions, was used vegetation material from experimental plants under inorganic N treatments ranging from N deficiency to excess. Foliar samples were collected from corn (*Zea Mays* L., in 2001 and 2004, at grain fill R3 reproductive stage), soybean (*Glycine max* (L.) Merr., in 2001) Illini cultivar and

maple (*Acer rubrum* L., in 2001). Photosynthetic efficiency measurements were collected *in situ*. The uppermost fully expanded leaves or 3<sup>rd</sup> leaf from terminal were excised from the plant canopy, immediately placed in water filled sample holders, and transported to the laboratory for spectral measurements, pigments and C and N determinations.

Corn samples were collected from an intensive test site for a multi-disciplinary project entitled "Optimizing Production Inputs for Economic and Environmental Enhancement" (OPE) to develop new farming strategies. The experimental design was a randomized complete block with treatment groups of 280 (150% of optimal), 140 (optimal, 100%), 70 (50%), and 0 (0%) kg N / ha, which provided a range of plant growth and condition. Measurements were acquired in August at the grain fill (R3) reproductive stage.

The soybean was grown in a sterile perlite growth medium where no rhizobium species were allowed to infect the roots. Nutrient solutions from 0.002M (N deficiency) to 0.005M (optimal plant growth) were applied weekly for 45 days. These levels correspond to 100%, 75%, 50%, 25%, and 0% of the nitrogen required for optimal growth. The plants were measured after eight weeks of growth.

Maple foliar samples were collected from a multiyear experiment where six-year old saplings of red maple were planted in the ground in 2001 and treated with N in the form of urea, with varying concentrations from 0.004 M to 0.001 M, simulating augmented levels of atmospheric N deposition.

## 2.2 Spectral measurements and data analysis

To determine the amount of F as a part of R, spectral measurements were conducted using procedures established by Kim et al. (1993) and further developed at by Zarco-Tejada (2000). High spectral resolution measurements were acquired using an ASD-FR FieldSpec® Pro, with 3nm FWHM and 1.4nm sampling interval in the 350-800nm region (Analytical Spectral Devices, Inc.; Boulder, CO). The sensor's foreoptic view angle was 8 degrees and the view area was approximately 2cm<sup>2</sup>. A spectrolon panel was used as a reference standard. A 300W xenon arc lamp and a set of neutral density filters (in 2001) and a 300W solar simulator (Oriel 91160A; Newport Stratford Inc., Stratford, CT) outfitted with Global Air Mass Filter (Oriel 81080; Newport Stratford Inc., Stratford, CT), were used to simulate constant solar radiation, resembling the spectrum in Washington, DC area in mid July (Figure 1). A Schott RG 665 long pass filter was used to prevent F induction from below 665nm. The illumination setup was used with the RG 665 filter blocking the light source to measure R, and without the filter to measure Ra (R+F, Figure 2 A). Samples were dark adapted for 5 minutes immediately before initiating the Ra measurements. Recording data every 0.01s for 25 seconds, spectra were obtained across the 650-800nm region.

ChlF was calculated as the difference between the R and Ra amounts (Figure 2 B):

$$F = Ra - R.$$

To test for significant differences in Fmax and Fs occurring among treatment levels associated with ChlF (at 685nm, 740nm and 760 nm), was conducted general linear model analysis of variances (GLM, ANOVA, SYSTAT 8.0; SPSS Inc., 1997). The significance of the differences was determined by Tukey-Kramer test. Estimates of the

attained maximum ChlF (F<sub>max</sub>) and the ChlF at steady state (F<sub>s</sub>), as a percentage of the incoming radiation, as a percentage of the radiation reflected from the vegetation (R) and in mW/m<sup>2</sup>/str/nm are given in Table 1.

Contemporaneously with the foliar spectra were acquired with measurements of photosynthesis, pigment concentration, carbon and N contents.

### 2.3 Biophysical Measurements

Photosynthetic capacity (A<sub>max</sub>,  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) was determined *in situ* with a Li-Cor 6400 photosynthetic system (Li-Cor, Lincoln, NE, USA) fitted with a leaf fluorometer chamber. A<sub>max</sub> was determined under controlled conditions of 2000  $\mu\text{mol m}^{-2} \text{ s}^{-1}$  PAR, saturating  $\text{CO}_2$  concentration (1000 ppm), controlled leaf temperature (25°C), and relative humidity (~35%). A<sub>max</sub> and light-adapted steady-state Chl F (F<sub>s</sub>) were obtained simultaneously in the field before excision of leaves for laboratory measurements.

To determine leaf Chl *a*, Chl *b*, and total carotene concentrations freshly cut leaf disks (2.54 cm<sup>2</sup>) were placed in 3.5 ml of dimethyl sulfoxide (DMSO) and sealed for 36 hours at 25°C. Absorption spectra were obtained using a dual beam spectrophotometer (Lambda 40, Perkin-Elmer, Norwalk CT, USA) and pigment concentrations were determined by procedures outlined by Wellburn (1998). The remainder of each leaf sample was oven dried at 50° C, and ground to pass a 1 mm mesh. Total leaf carbon (C) and N determinations were obtained by the Dumas combustion method (Bellomonte et al., 1987).

### 3. RESULTS AND DISCUSSION

In the red-edge region vegetation has relatively low R (Figure 2A), due to a strong chlorophyll *a* absorption. In all cases the radiation measured without the long pass filter (Ra) was consistently higher than R measured with filter, due to the foliar ChlF emissions (Figures 2, 4 and 6). Although the absolute ChlF amounts were relatively small, Fs contributed significantly to Ra. The time required for reaching from Fmax to Fs (Figures 3 and 4) varied within 15 to 20 seconds, which was not found significantly associated with either species type or N treatment level.

#### Variation in ChlF with species type

The relative contribution of ChlF to the Ra at each wavelength (Fmax and Fs) varied significantly among species, with the maple providing significantly higher emissions than both corn and soybean. (ChlF expressed as a proportion of Ra, Fig. 5). At 685 nm the ChlF contribution was in the range of 10-50 % of the Ra, at 740 nm it amounted to 3-20 % and at 760 nm was 2-18% (Table 1, Figure 5). These amounts of ChlF agree with the range of values reported for variety of species under solar illumination conditions (Corp et al., 2006; Dobrowski et al., 2005; Louis et al., 2005; Meroni et al., 2006; Theisen, 2000). Fs and its ratios consistently allowed for species separation when Fmax did not (Table 1).

The red/far-red ratio, using F740s (Table 1) allowed for species separation, which agreed with earlier studies using single blue, green or red excited F (Chappelle et al., 1999; Corp et al., 1996; Middleton et al., 1996). When using the F760s, located at the shoulder of the spectral curve, the Fs intensity and the sensitivity of the red/far-red ratio

were much lower and species separation was not possible (Table 1). While the red/far-red ratio measured using near solar excitation can provide similar results to the one obtained using the established excited F procedures, its sensitivity sharply decreases if the far-red Fs is collected away from the F peak at 760 nm (the location of prominent telluric O<sub>2</sub> feature of interest for instrument development).

The shape of the spectra in relative units differs significantly from the spectra expressed in absolute units and (Figure 4). Some of the variations in the spectra when expressed in percentage of the incoming radiation (as is common for R measurements) could be associated with the quality of the excitation light. Using solar simulator as an excitation source, in the current study we used close to constant solar illumination (Figure 1), however some of the spectral differences remain, as does the need to validate for various solar conditions the previously established, using single excitation band, Fs trends and relationships of Fs to photosynthetic function and vegetation stress.

#### **Variation in ChlF with N treatment in corn**

Based on both 2001 and 2004 data (Table 2), on average 10-25%, at 685nm and 2-6% at 740nm of Ra was due to ChlF, which comply with the range in solar induced ChlF reported by Corp et al. (2006) for corn under N treatments, using a different measurement approach. The amount of Fs varied in association with the N treatments, and significantly affected the relationship between foliar ChlF and R (Figure 6).

A slight decreasing trend was observed in Fmax in association with N deficiency (Figure 7 A), while the relative Fs fraction of the apparent R increased in concert with the nutrient stress levels (Fig. 7 B, Table 2). Optimal separation of the N treatments was

achieved by F685s, (Table 2). Using both 2001 and 2004 corn data, F685 allowed consistently for treatment separation (Table 2). The variation in corn ChlF intensity associated with the N treatments was lower than the variation associated with the different species.

Using active F techniques the red /far-red ChlF ratio has been shown to increase in association with vegetation stress, as chlorophyll content decreases or as photosynthetic rate declines (Chappelle et al., 1999; Lichtenthaler & Rinderle, 1988; McMurtrey et al., 1994; Middleton et al., 2005; Theisen et al. 1994). The pattern observed in the steady state F685/F740 ratio (Figure 8), complied well with the trends suggested in the literature with advancement of vegetation stress resulting from N deficiency or excess N supply.

The well established induction kinetics parameters – Fvs and Fvm, were calculated using the measured Fmax and Fs. Maximal Fvs and Fvm were observed from the optimal corn treatments (N=100%, Figure 9). The trends in the produced Fvs and Fvm (Figure 9) comply with the reports of previous studies (Lichtenthaler et al., 1986; Mohammed et al., 1995) and illustrate the utility of the current measurement approach for achieving both steady state ChlF and induction kinetics parameters. Both Fvs and Fvm (Fig. 9) have been suggested as indicators of photosynthetic efficiency and function (Lichtenthaler & Rinderle, 1988; Lichtenthaler et al., 1986). Our finding of Fvs values of <2.5 for N deficiency (0% and 50%, Fig. 9), could be an indicator of an inefficiency in the carbon dioxide assimilation associated with N deficiency (Mohammed et al., 1995).

#### 4. CONCLUSIONS

This investigation confirms for a number of species the significant contribution of ChlF to the apparent vegetation R in the red and far-red regions. On average, 10-25% of Ra at 685 nm (Fs) and 2-6% (Fs) at 740nm was actually due to ChlF. At 685nm Fmax was ~2-4% and Fs 0.5-1.5% of the incoming radiation, while at 740nm the ChlF values were higher: Fmax ~ 4-6%, Fs ~1-2%. The relative steady ChlF fraction at 685nm increased in concert with the nutrient deficiency levels. The relationship between foliar F and R was significantly affected by the physiological status of the vegetation, for corn under N treatments.

Using a simulated solar excitation spectrum, this study confirms the trends in F685/F740s, Fvs and Fvm ratios associated with vegetation stress, previously established using a narrow spectral excitation band. This finding suggests that the ChlF utility for monitoring the physiological status of vegetation, established by the active ChlF technology is likely to provide results comparable to natural solar illumination.

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## Figures

Fig. 1. Solar spectra and radiation output of the Xenon lamp (300W, 2001) and Solar simulator (300W, 2004), used as reflectance illumination and ChlF excitation sources.

Fig. 2: Reflectance (A, R solid line), apparent reflectance (A, Ra dashed line) and fluorescence (B,  $F_s = Ra - R$ ). Data from corn foliage (2001, ANOVA, LS Means).

Fig. 3. Time resolved Chlorophyll Fluorescence spectra ChlF (% of R) of corn foliage (*Zea Mays L.*). The spectra was acquired after 5 minutes of dark adaptation of the samples at 0.01 seconds interval.

Fig. 4. Maximum and steady state ChlF levels for corn foliage (*Zea Mays L.*, Means and LS Standard Errors). The spectra were acquired after 5 minutes dark adaptation of the samples at 0.01 second intervals. F levels decreased significantly from Fmax (black line) to  $F_s$  (grey line) in 15-20 seconds.

Fig. 5. Maximum and steady state ChlF for maple, corn and soybean (Means and Standard Errors).

Fig. 6. While the contribution of  $F_s$  to R may not appear significant (A), it may account for about 15% of the reflectance intensity (B). R (solid lines) and Ra (dashed lines) for

healthy (N = 100%, black lines) and nitrogen deficient (N = 0%, grey lines) corn foliage (corn 2001, ANOVA, LS Means).

Fig. 7. Maximum (A) and steady state (B) ChlF from nitrogen deficient (N=0%, grey lines) and healthy (N=100%, black lines) corn foliage (corn, 2004 data,ANOVA LS Means).

Fig. 8. ChlF measured under full solar spectrum excitation can be indicative of N availability (corn): Differences in the F red/far-red ratio associated with nitrogen treatments (ANOVA, LS Means and Std. Errors).

Fig. 9. Differences in the Fvs [ $F_{685vs} = (F_{max} - F_s) / F_s$ ] and Fvm [ $F_{685vm} = (F_{max} - F_s) / F_{max}$ ] parameters in association with nitrogen treatment. The Fmax and Fs, measured under a full solar spectrum, show relevance to the well established induction F parameters, such as Fvs and Fvm.

## Tables

Table 1. Changes in ChlF parameters in maple, corn and soybean

Table 2. Changes in ChlF parameters in corn (2001 and 2004) associated with N treatment

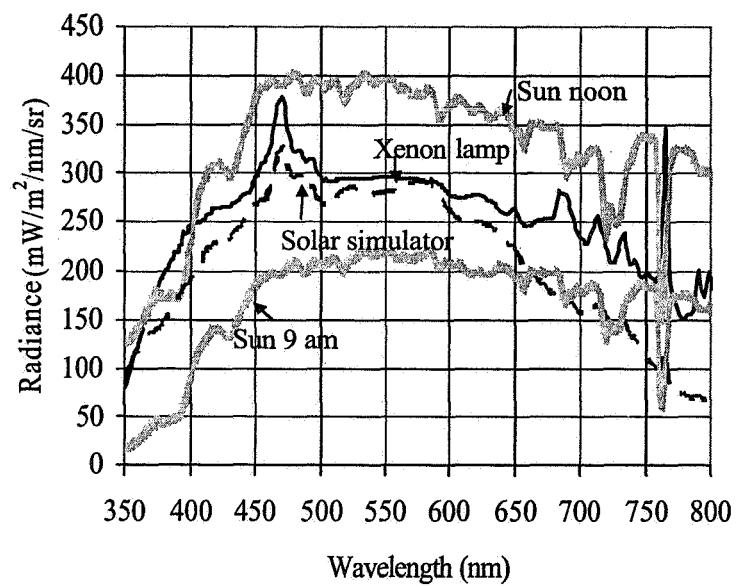


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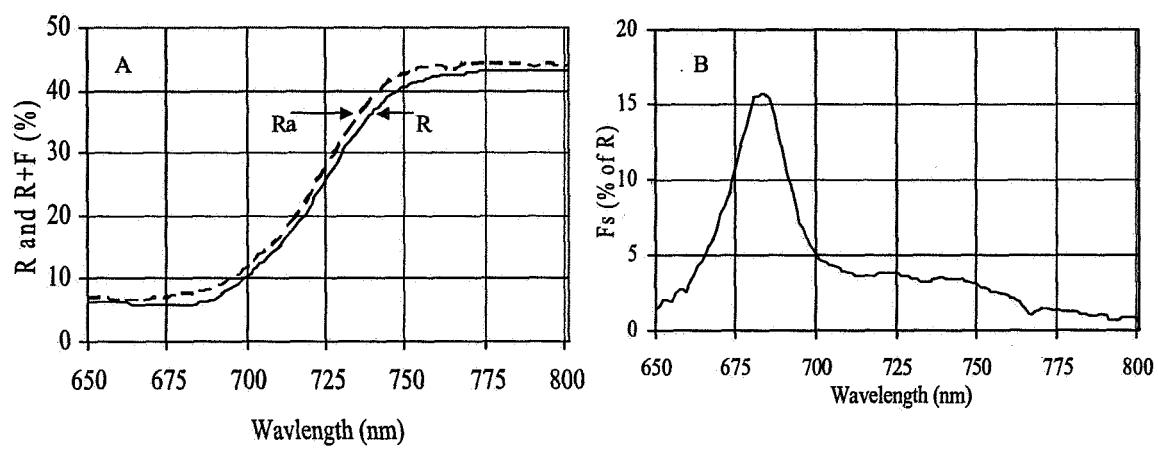


Fig. 2. Reflectance (A, R solid line), apparent reflectance (A, Ra dashed line) and fluorescence (B,  $F_s = Ra - R$ ). Data from corn foliage (2001, ANOVA, LS Means).

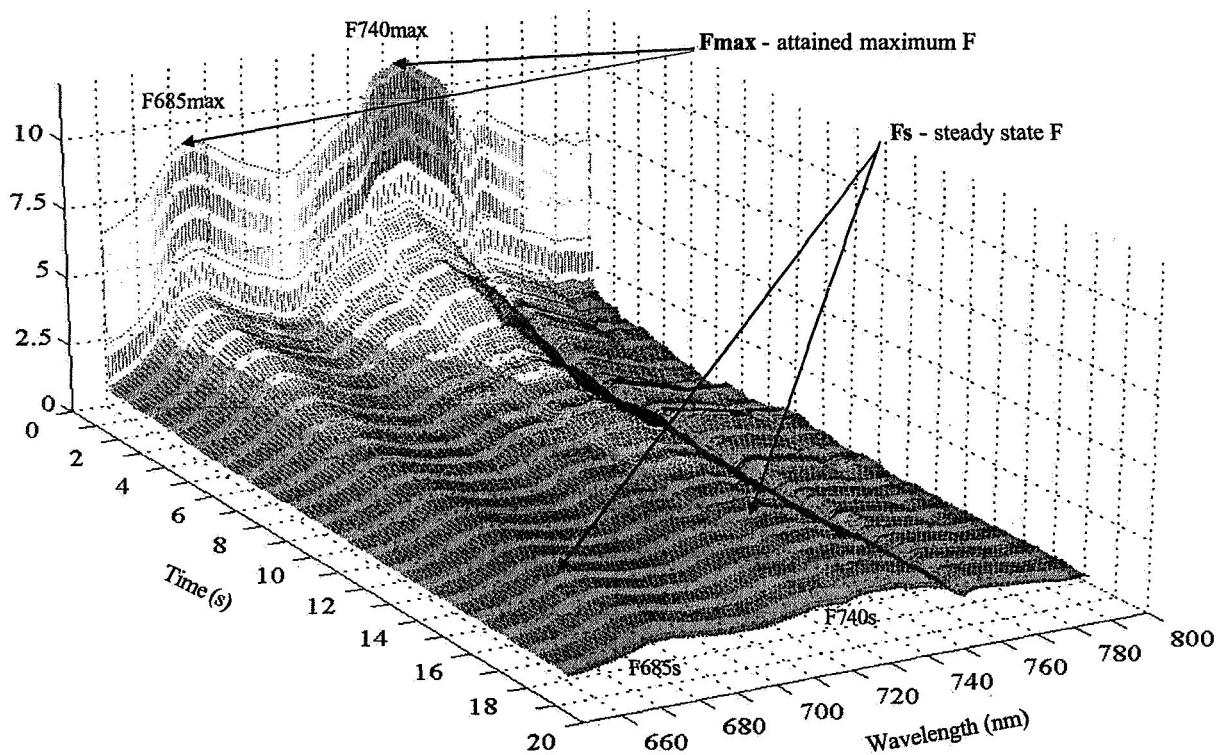


Fig. 3. Time resolved Chlorophyll Fluorescence spectra ChF (% of R) of corn foliage (*Zea Mays L.*). The spectra was acquired after 5 minutes of dark adaptation of the samples at 0.01 seconds interval.

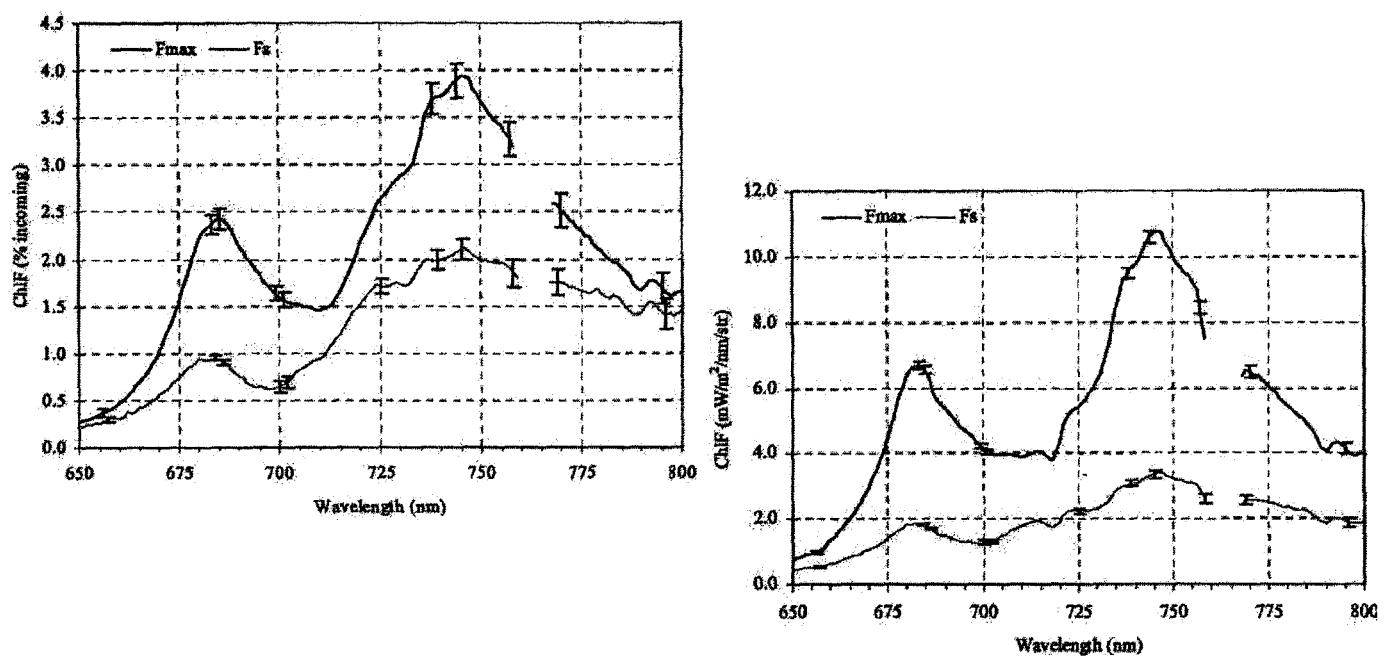


Fig. 4. Maximum and steady state ChlF levels for corn foliage (*Zea Mays L.*, Means and LS Standard Errors). The spectra were acquired after 5 minutes dark adaptation of the samples at 0.01 second intervals. F levels decreased significantly from Fmax (black line) to Fs (grey line) in 15-20 seconds.

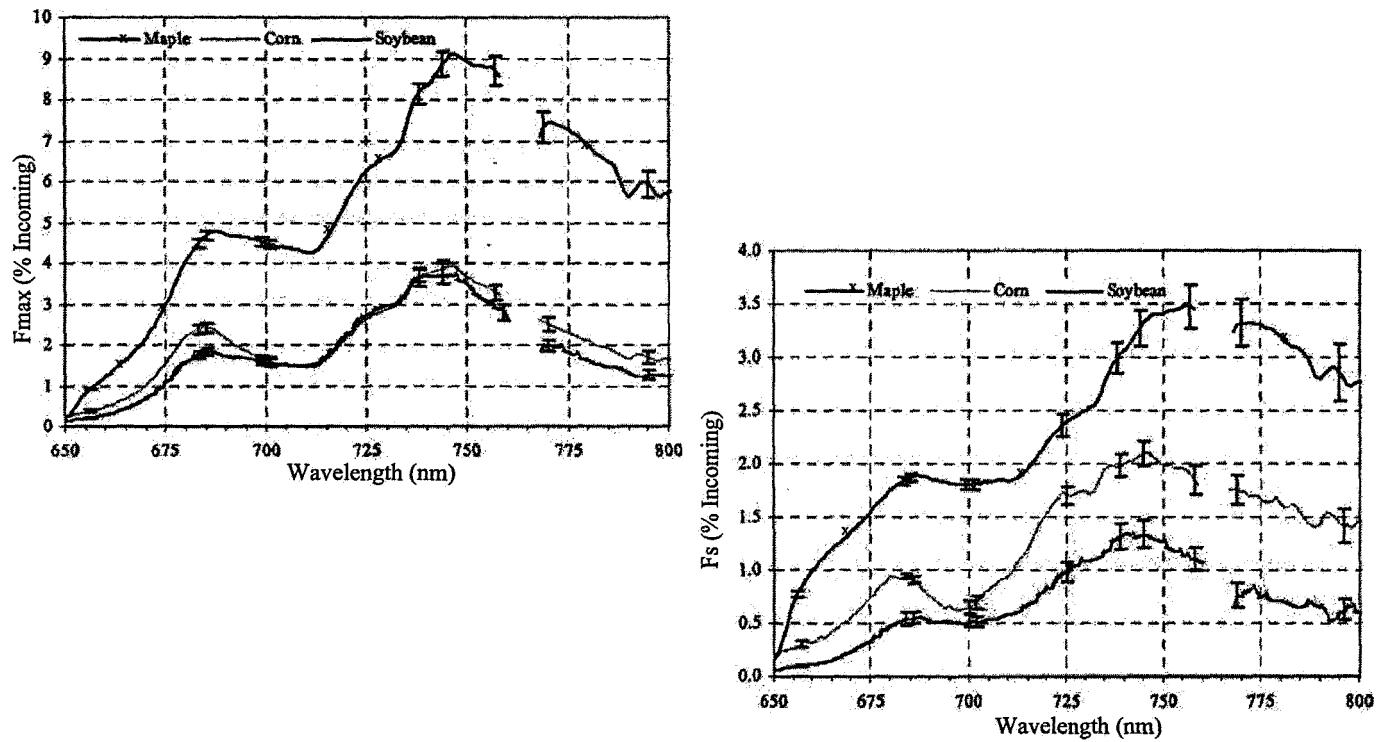


Fig. 5. Maximum and steady state ChlF for maple, corn and soybean (Means and Standard Errors).

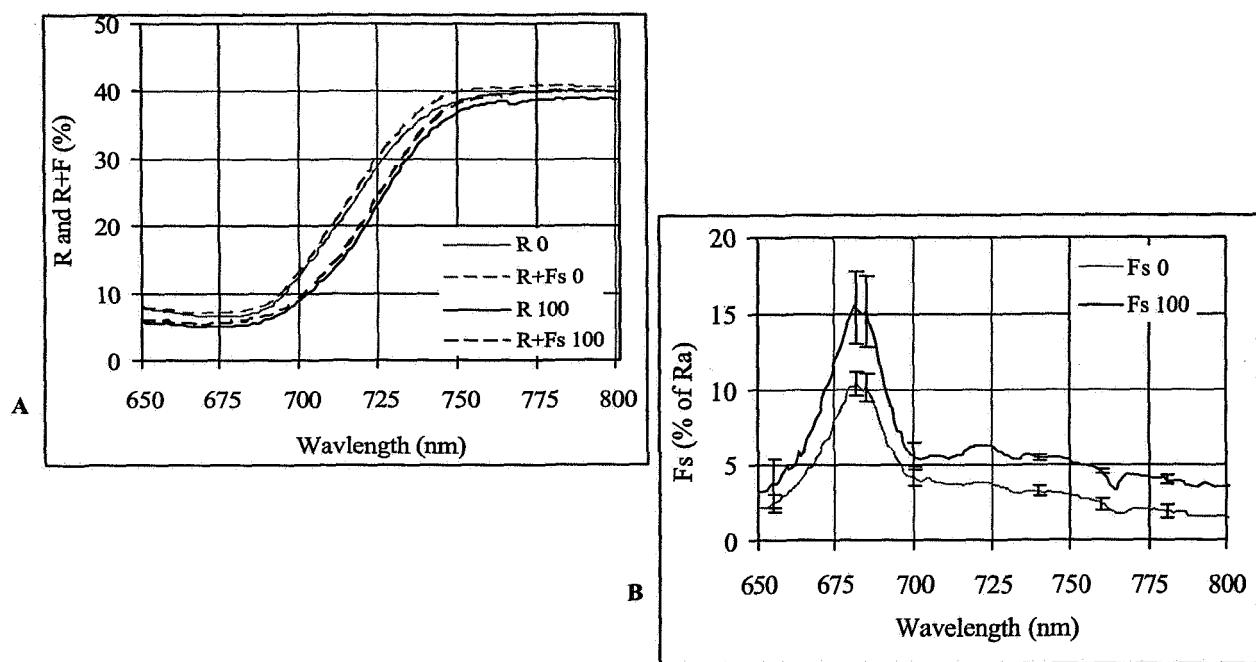


Fig. 6. While the contribution of  $F_s$  to  $R$  may not appear significant (A), it may account for about 15% of the reflectance intensity (B).  $R$  (solid lines) and  $R_a$  (dashed lines) for healthy ( $N = 100\%$ , black lines) and nitrogen deficient ( $N = 0\%$ , grey lines) corn foliage (corn 2001, ANOVA, LS Means).

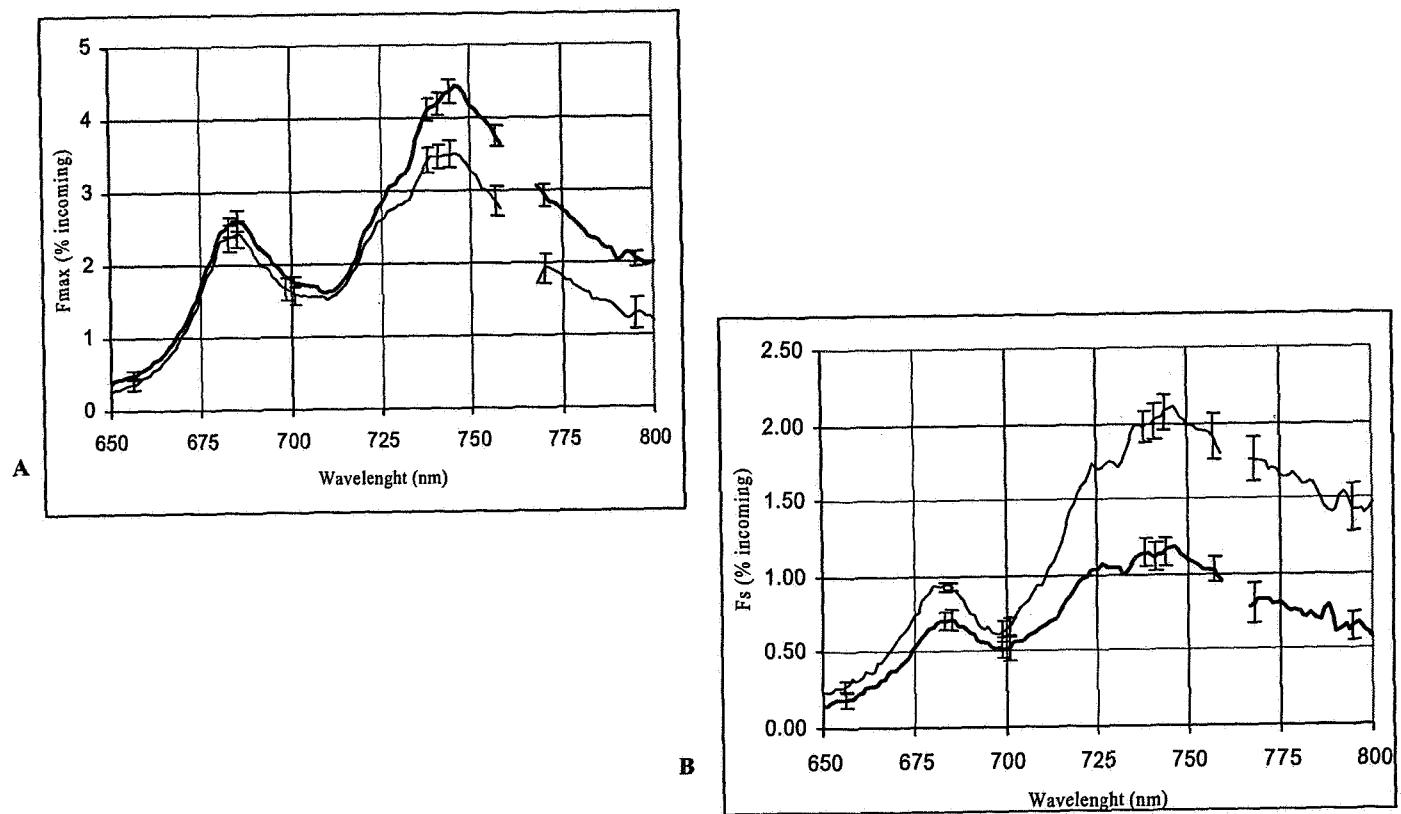


Fig. 7. Maximum (A) and steady state (B) ChlF from nitrogen deficient (N=0%, grey lines) and healthy (N=100%, black lines) corn foliage (corn, 2004 data,ANOVA LS Means).

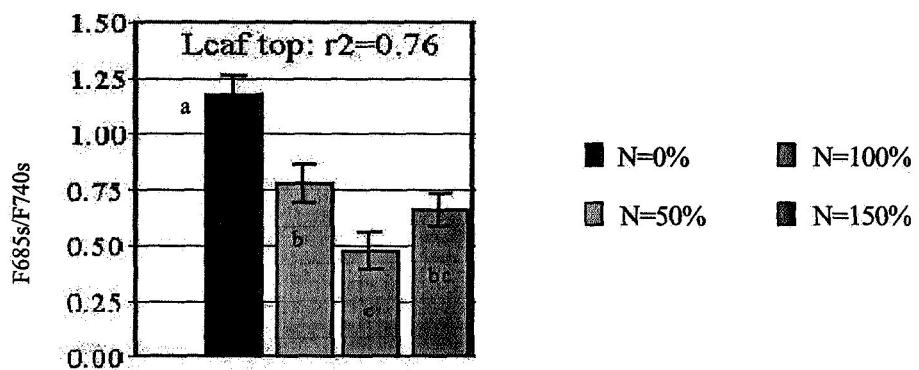


Fig. 8. ChlF measured under full solar spectrum excitation can be indicative of N availability (corn): Differences in the F red/far-red ratio associated with nitrogen treatments (ANOVA, LS Means and Std. Errors).

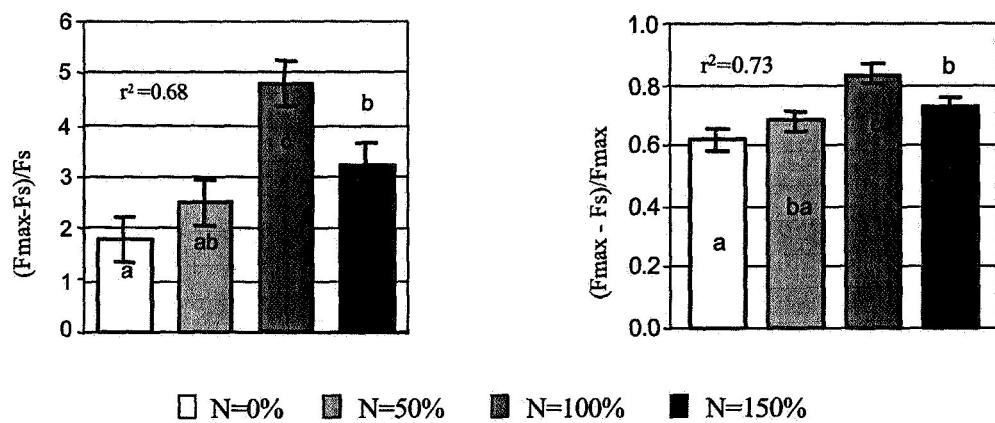


Fig. 9. Differences in the Fvs [ $F_{685vs}=(F_{max}-Fs)/Fs$ ] and Fvm [ $F_{685vm}=(F_{max}-Fs)/F_{max}$ ] parameters in association with nitrogen treatment. The  $F_{max}$  and  $Fs$ , measured under a full solar spectrum, show relevance to the well established induction F parameters, such as Fvs and Fvm.

**Table 1. Changes in ChlF parameters in maple, corn and soybean**

Fluorescence parameter †	Species		
	Maple	Corn	Soybean
<b>1. Fluorescence amount at 685 and 740 nm expressed as % of the incoming radiation</b>			
F685max	4.58a	2.43b	1.82c
F685s	1.87a	0.95b	0.54c
F740max	8.34a	3.73b	3.70b
F740s	3.05a	1.99b	1.34c
F760max	8.57a	3.19b	2.69b
F760s	3.46a	1.80b	1.12c
F685s/F740s	0.61a	0.49b	0.40c
F685s/F760s	0.54a	0.53a	0.48a
<b>2. Fluorescence amount at 685 and 740 nm expressed as % of radiation reflected of the vegetation</b>			
F685max	57.32	39.56	34.75
F685s	32.2	12.25	10.95
F740max	21.56	10.38	8.92
F740s	7.74	3.58	2.93
F760max	18.28	7.23	5.77
F760s	7.56	2.96	2.05
<b>3. Fluorescence amount at 685 and 740 nm (mW/m<sup>2</sup>/sr/nm)</b>			
F685max	12.63	6.55	4.91
F685s	5.04	1.81	1.81
F740max	21.79	9.74	9.66
F740s	7.98	3.09	3.51
F760max	20.19	7.51	6.75
F760s	8.15	2.61	2.62

† Means are compared within row per leaf surface, indicating significant differences among treatments with different letters (ANOVA)

**Table 2. Changes in ChlF parameters in corn (2001 and 2004) associated with N treatment**

Fluorescence parameter † (F)	Nitrogen treatment levels ‡				
	0	50	100	150	$r^2$
<b>1. F at 685 and 740 nm expressed as % of the incoming radiation</b>					
F685max (2001)	2.98ns	2.65ns	2.51ns	2.65ns	0.55
F685max (2004)	3.92b	3.91b	4.08b	4.95a	0.72
F685s (2001)	1.07a	0.79b	0.41c	0.67b	0.74
F685s (2004)	1.68b	2.23a	0.85c	1.96b	0.72
F740max (2001)	3.48a	4.08bc	3.91b	4.40c	0.61
F740max (2004)	6.91c	7.44b	7.71b	12.73a	0.69
F740s (2001)	1.02a	1.04a	0.78b	1.03a	0.62
F740s (2004)	3.64b	4.70a	2.75c	3.65b	0.75
<b>2. F at 685 and 740 nm expressed as % of radiation reflected of the vegetation</b>					
F685max (2001)	35.81	30.83	46.70	44.90	
F685max (2004)	47.11	45.49	63.30	72.82	
F685s (2001)	15.20	11.03	10.05	12.80	
F685s (2004)	23.87	31.14	20.84	37.44	
F740max (2001)	9.50	8.80	11.40	12.00	
F740max (2004)	18.86	16.05	22.48	34.72	
F740s (2001)	5.50	4.50	3.10	3.30	
F740s (2004)	19.63	20.34	10.93	11.69	
<b>3. F at 685 and 740 nm (mW/m<sup>2</sup>/sr/nm)</b>					
F685max (2001)	5.10	4.32	5.51	5.31	
F685max (2004)	6.71	6.37	8.96	9.92	
F685s (2001)	2.02	1.40	1.32	1.51	
F685s (2004)	3.17	3.95	2.74	4.42	
F740max (2001)	9.63	8.71	10.57	11.62	
F740max (2004)	19.12	15.88	20.84	33.24	
F740s (2001)	5.38	4.32	2.91	3.26	
F740s (2004)	19.20	19.34	10.26	11.55	

† Means are compared within row per leaf surface, indicating significant differences among treatments with different letters (ANOVA)

‡ Nitrogen treatment levels (% of optimum, LS Means)